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From Canaries to Cats: Domestic Animals as Sentinels for Human Exposure Effects

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Equine veterinarian Kathleen Mullen has fielded her fair share of strange calls. So when she received a question about some foals in Pennsylvania that were having difficulties swallowing, it seemed all part of a day's work. Issues with swallowing—known as dysphagia—affect about 1 in 100 foals,[1] but on this farm 5 of 10 foals were affected. She first thought the problem might be genetic, but close relatives of the affected horses living 420 km east, in New York, had shown no signs of dysphagia. So Mullen began looking for an environmental cause.[2]

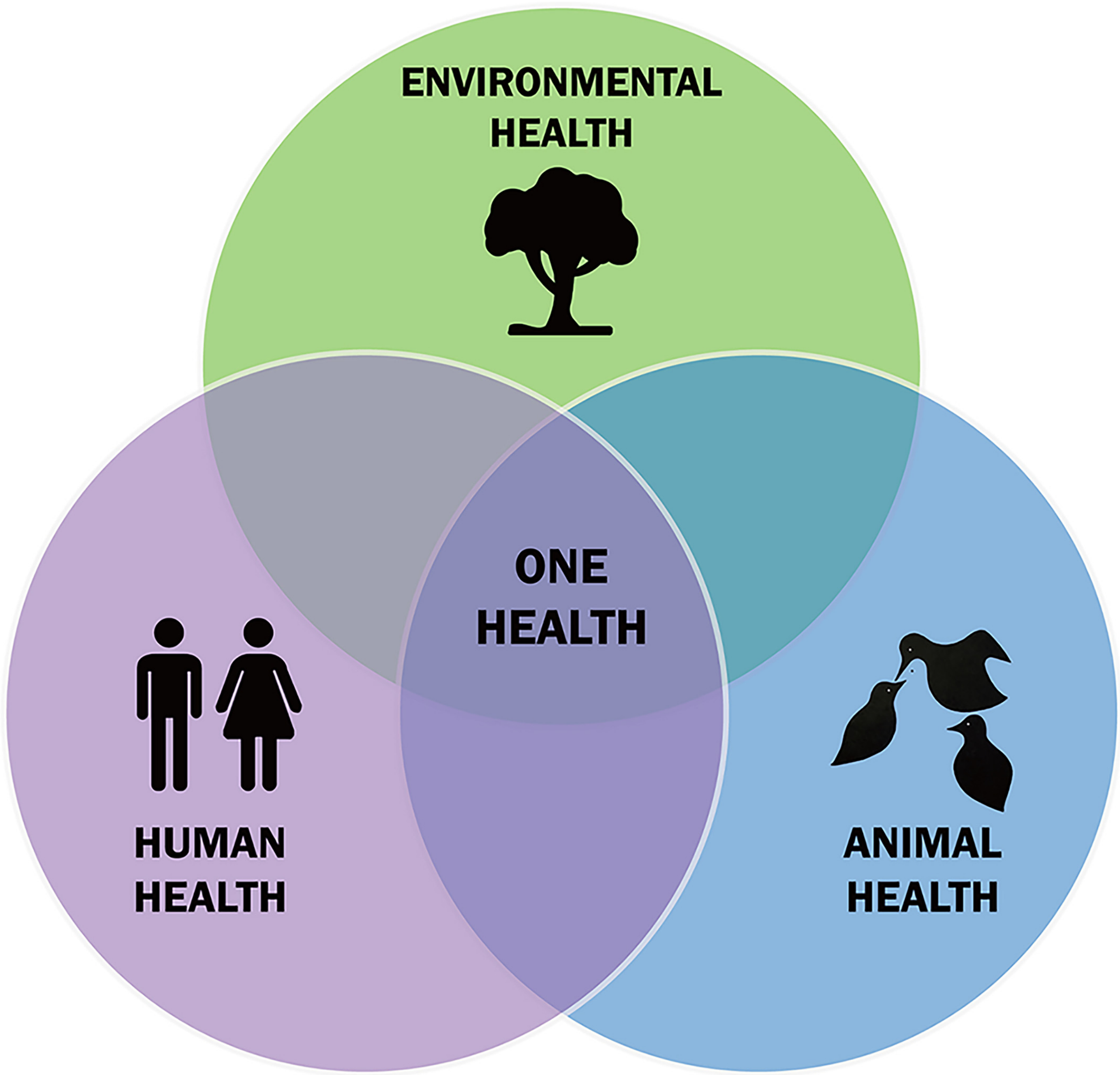
As she dug into the issue, Mullen learned that drinking water for the Pennsylvania farm was drawn from wells approximately 500 m from a hydraulic fracturing (fracking) well, whereas there was no drilling activity near the New York farm. She knew that chemicals reportedly used during fracking activities have shown endocrine-disrupting[3,4] and developmental impacts[5] and can affect sensory organs, as well as respiratory and gastrointestinal systems.[6] Furthermore, polycyclic aromatic hydrocarbons, which are emitted during natural gas extraction, have been found in water and air near fracking sites.[7] But to determine whether there was any connection between the sick foals and the fracking operation, Mullen and her colleagues needed to understand connections between potential exposures and dysphagia risk.

Clinical and laboratory data were obtained from mares and the 65 foals—both dysphagic and unaffected—born at the two farms during the study period. After ruling out known causes of dysphagia in foals (such as anatomical malformations and prematurity), the team found[2] that the odds of a foal developing dysphagia increased 1.4 times for each month of pregnancy its mother had spent on the Pennsylvania farm, compared with the New York horses. The association was especially strong in male foals, whose risk was 5.5 times higher than the females born at the Pennsylvania farm. Analyses of soil, air, feed, and water samples from both farms showed that concentrations of four polycyclic aromatic hydrocarbons associated with fracking were higher in the Pennsylvania water samples than those from New York. After an improved water filtration and treatment system was installed at the Pennsylvania farm, no more dysphagic foals were born.

"This research was a great example of studying an animal species as a sentinel for potential health risks for humans," Mullen says. "It is really important to consider animals for this role. We live very closely with them; often they share our houses. There is a lot of potential to look at linkages among animal health, human health, and the environment." This holistic approach is known as One Health (https://www.cdc.gov/onehealth/index.html).

House pets, such as cats and dogs, share humans' mammalian biology, as well as similar exposures to chemicals in water, furnishings, air, and sometimes food (most pets do not share their owners' exposures outside the home, such as those at work or school). Pets have shorter life spans than humans, thus the time from an environmental exposure to the development of an

Standardbred foals, like the one shown above, were affected by dysphagia at a Pennsylvania farm located near a fracking well. No more affected foals were born after new water filtration technology was installed at the farm. Image: © D. Cribbie/Shutterstock.com.



The One Health approach recognizes the interconnections between people, animals, and the environment, including plants, animals, water, and climate. Image: Thddbfk, via Wikimedia Commons, used under CC BY-SA 4.0 license. https://creativecommons.org/licenses/by-sa/4.0.

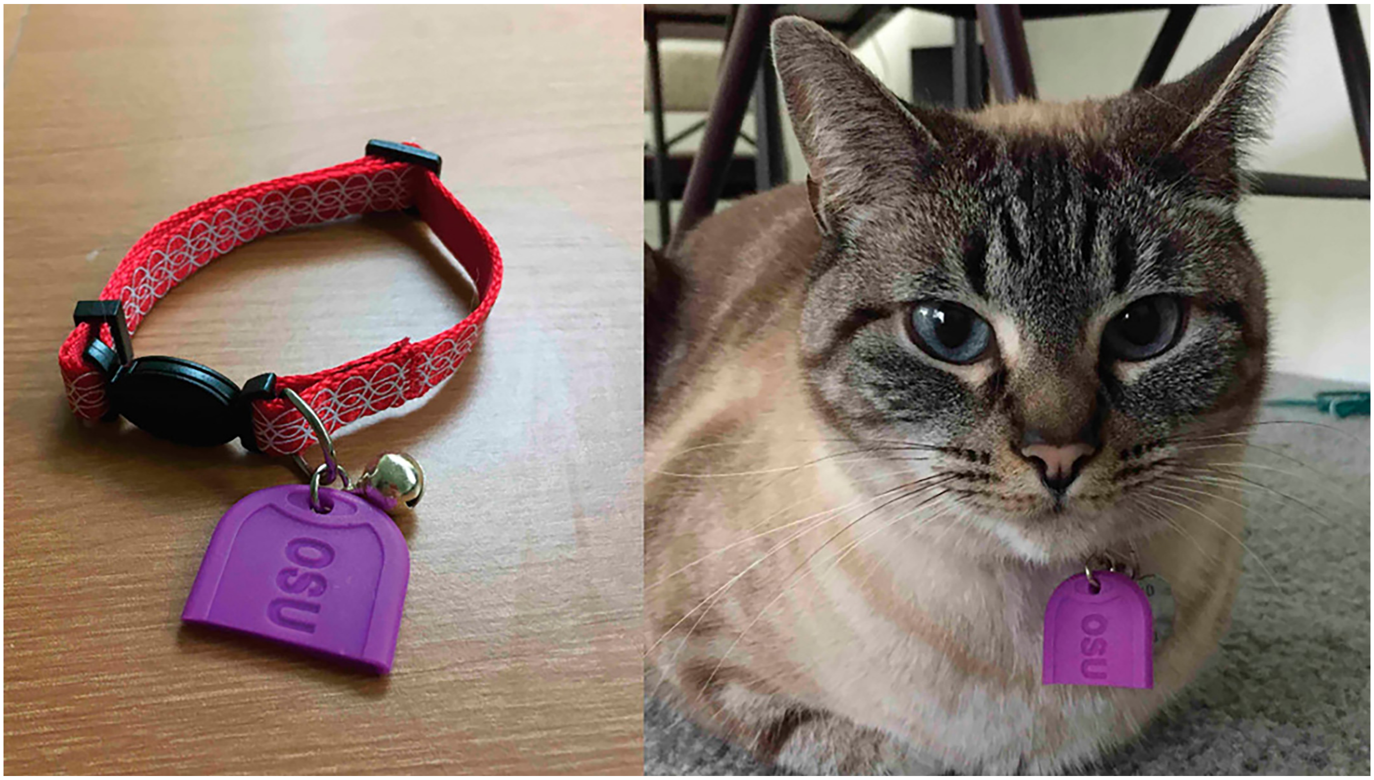
associated disease is highly accelerated, on the order of years instead of decades.[8] Given those shared environmental exposures, an animal's exposure-related disease might presage subsequent disease in its owner. A better understanding of these links could facilitate enhanced screening and other preventive measures.[9] So environmental health scientists are now studying dogs, cats, horses, and other domestic animals as sentinel species, to discover early warning signs of potential adverse effects on human health.[10]

"It is no longer the canary in the coal mine, but the dog in our bed or the cat on the couch," says Audrey Ruple, a veterinarian and bioinformatics specialist at the Virginia-Maryland College of Veterinary Medicine. "I really want to [study] animals and leverage the data to help human populations use them as an early indicator."

Matthew Breen, a research scientist at North Carolina State University, says this strategy is emerging as a potentially powerful way to improve the health of both humans and their animal companions. "If there is an environmental risk factor that could be affecting my dog, potentially, how would it impact me?" asks Breen, who is studying whether bladder cancer in dogs might be useful for predicting an increased risk of the same disease in their human owners.

Cats Contribute to Exposure Research

Some of the earliest studies on animals' environmental exposures involved feline hyperthyroidism, which was first described in 1979.[11,12] By the mid- to late-1980s, veterinarians across the United States were seeing an influx of cases.[13] Cats, many in the later stages of life, were showing up at clinics emaciated and hyperactive, with diarrhea and oily, unkempt fur, unable to keep weight



Silicone tags, adapted from wristbands worn for human exposure assessments, are finding wider use in environmental health research, including as collar tags to test exposures in cats and dogs. Image: Reprinted with permission.[14] Copyright 2019 American Chemical Society.

on no matter how much they ate. Between 1980 and 2014, the incidence of hyperthyroidism in older cats increased from 1 in 200 to 1 in 10.[14] The sudden rise in cases, combined with the genetic heterogeneity of most domestic cat populations, suggested an environmental factor might be involved.[13]

By the early 2000s, environmental health researchers were taking a closer look at a class of chemicals called polybrominated diphenyl ethers (PBDEs).[15] Used as flame retardants in everything from children's pajamas to upholstery fabric, PBDEs surged in popularity beginning in the 1970s.[16] Epidemiological research in cats found a strong association between PBDE levels in homes and feline hyperthyroidism, but investigators could not confirm whether cats with the highest PBDE exposures were also at the highest risk for developing hyperthyroidism—a key missing link.[17] However, along with experimental studies in mice,[18] these findings did provide a clear link between PBDE exposure and thyroid problems. Environmental health scientists, such as Janice Dye at the U.S. Environmental Protection Agency (EPA), wondered whether the thyroid problems vets were seeing in cats could be a harbinger for human issues related to the long-lived compounds, such as the human equivalent of feline hyperthyroidism, called toxic nodular goiter.[19] The challenge for researchers, Dye says, was finding a method to measure chemical exposure in cats without creating undue stress for the pets, their owners, or scientists.

Meanwhile, toxicologist Kim Anderson at Oregon State University was developing silicone wristbands that could be worn to collect data on human exposures. The silicone matrix passively absorbs airborne chemicals during the wearer's daily activities at home and work.[20] The silicone bands are cheap, easy to use, and able to provide answers relatively quickly. Experiments confirmed that humans' dermal and inhalational exposures to semivolatile chemicals as reflected by the wristbands corresponded with measurements gathered by personal active air samplers, such as those worn as backpacks.[21] The wristband levels also correlated well with biomarkers and metabolites found in serum[22] and urine,[23] outperforming exposure sampling via hand wipes.[24]

Using similar silicone collars or tags for cats could help scientists explore the feline hyperthyroidism mystery, Anderson realized. In 2018, she reported preliminary evidence from a 1-week pilot study of three cats that showed exposures to 11 PBDEs and organophosphate ether flame retardants.[25] A subsequent study of 78 adult cats (half of which had hyperthyroidism) found that, among the 44 flame retardants studied, concentrations of tris(1,3-dichloro-2-isopropyl) phosphate were most strongly associated with hyperthyroidism and other potentially harmful changes in thyroid hormone levels.[14]

Animal Genomes Open Doors to Cancer Research

Anderson's silicone tag findings proved a major advance in understanding the health impacts of environmental exposures in both humans and their pets. This advance occurred alongside two other events: the genomics revolution, which enabled fast, cheap DNA sequencing,[26] and a rise in the monetary investments people were making in their pets.[27] Research into diseases shared between dogs and humans really took off in 2005, with the first publication of the complete dog genome sequence.[28] The cat and horse genomes followed in 2007[29] and 2009,[30] respectively. Investigators, including Breen and veterinary oncologist Rodney Page at Colorado State University, began using next-generation sequencing, comparative genomics, and bioinformatics to understand the intricacies of tumor biology in dogs.[31]

"Tumors in dogs and humans look very similar under the microscope, and they are very similar clinically," says Page. "One of the opportunities afforded by genomic sequencing is looking at the nature of the way the tumors may have started and what the dogs might have been exposed to environmentally to ultimately identify potential preventive strategies."

The West Highland white terrier, left, and Jack Russell terrier, right, are among the dog breeds with increased risk of urothelial carcinoma, a cancer of the urinary tract, according to Breen. Image: © LanKS/Shutterstock.com.

Breen sees these close similarities in certain types of tumors as a way to understand the biological drivers of various cancers. Rather than study just the parallels in innate tumor biology, Breen began to consider what environmental health scientists were learning about exposures, the disruption of underlying molecular biological pathways, and the development of cancer. Making these connections in humans, he knew, was challenging, even in the best of circumstances. Human cancers may develop over decades, such that a tumor identified in a person's seventh or eighth decade may be the result of environmental exposures in childhood—or even *in utero*.[32] Moreover, most of us have little knowledge about the forever-shifting chemical soup of our environment. Even if we knew the precise distance between, say, our childhood home and an oil refinery or freeway, we could not be certain about the types and levels of exposures that occurred.

Breen's preliminary work showed that 85% of urogenital cancers in dogs had a specific mutation in a gene called the *BRAF* gene.[33] He has teamed up with Duke University professor Heather Stapleton and her postdoctoral fellow Catherine Wise to investigate whether humans and dogs with bladder cancer might share certain environmental exposures.[34,35] "We are spending $6–7 billion dollars a year on cancer research, and people are still succumbing to the disease," Breen says. "If we keep looking for answers [just] within our own genome, I think we will be waiting a long time."

Meanwhile, the Dog Aging Project is enrolling tens of thousands of dogs across the United States and following them across their life spans to investigate the canine aging process.[36] Researchers are studying genetic and environmental factors that contribute to aging in general and to associated diseases, such as cancer, says Ruple, who is affiliated with the project. Identifying these factors in dogs could give scientists who study aging in humans some clues about where to look, she says.

Moving the Field Forward

At England's University of Nottingham, veterinary scientist Rebecca Blanchard is investigating reproductive problems that have begun to crop up in stallions[37] and declines in sperm quality and increases in testicular cancer incidence in both humans and dogs. She and her team analyzed canine testicles removed during routine neutering and found that tissue levels of diethylhexyl phthalate (DEHP), PBDEs and polychlorinated biphenyls (PCBs) differed across locales in England, Finland, and Denmark.[38] Testes from dogs in Finland and Denmark had higher concentrations of PBDEs and lower concentrations of DEHP and PCBs than those from dogs in England, along with lower levels of certain cellular pathologies. Blanchard is not yet sure whether the differences are linked to specific chemicals or exposure levels, but she is finding links between declines in the quality of the dogs' sperm and exposure to DEHP and PCBs.[39] Her findings may help scientists understand certain fertility problems in humans, such as reduced sperm count and motility.[40]

Young children and pets spend more time on the floor than adults and older children and thus have greater proximity to house dust, say Dye and other researchers. Image: © Bakhrom/Shutterstock.com.

Meanwhile, researchers such as Dye from the EPA and others have begun studying exposures of adults, children, and cats to per- and polyfluoroalkyl substances (PFAS) in the home.[41] "Pet owners and parents have been targeted to help keep their homes stain-free by using stain-resistant coatings and other products containing PFAS on their furniture and carpeting," Dye says. "It was amazing how the [levels] of chemicals in some of our [most highly exposed] cats and kids were similar in both total magnitude and relative amounts." In a previous study on flame retardants,[42] Dye and colleagues found closer similarities in dust ingestion between cats and toddlers than between cats and adults, possibly due to the children's mouthing behavior and proximity to the floor.

Mullen's studies on the sick foals in Pennsylvania suggested that steps to improve the health of horses—water filtration in that case—might also improve human health. But one of the biggest challenges to using domestic animals for human health surveillance is connecting veterinary records with human medical records, she says. Coordinating human medical records between health care systems is itself a major challenge, according to Mullen, and it would be even more complicated to add in veterinary platforms.

The lack of data is a huge barrier, agrees Ruple. "You have incredible health data sets for large animal species from the U.S. Department of Agriculture," she says, noting there is no similar data set for dogs and cats—the animals with life experiences most like ours.

In addition, Ruple says, the data that do exist are not representative of the whole pet population. She points out that even large, population-based studies, such as the Dog Aging Project, may be skewed toward wealthier families with more resources to spend on their pets, including the time and energy to enroll their dogs in a research study. This means that studies like the Dog Aging Project might unwittingly focus on environmental exposures relevant to pets and people in higher-income households, potentially overlooking factors more pertinent to those in lower-income households. "I worry about creating even a greater divide in the sociodemographic and racial disparities that we see in human health," Ruple says.

Anne Thessen, a data information specialist at the University of Colorado, points out that scientists cannot rely solely on links between environmental exposures and disease in pets to say which exposures are driving disease in humans. "The major limitation here, of course, is that these studies are all just hypothesis generation," she says. "You do not want to take the findings at face value; they are something to take to the lab to help you get to answers faster."

This young field is rapidly attracting more interest—and more grant funding from government agencies—says Thessen. With so many of us living side by side with pets, emerging knowledge on how the environment affects everyone in it promises to benefit both humans and pets, she says.

Part of that driving force, according to Breen, is people's love for their pets. He says dog owners, for example, feel part of a community. “It is not about *my* dog, it is about *all* our dogs,” he says. “I think that is why we have an opportunity to use that community spirit in a very productive way.”

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